INTRACAPSULAR DEVELOPMENT OF *THAIS HAEMASTOMA CANALICULATA* (GRAY) (PROSOBRANCHIA: MURICIDAE) UNDER LABORATORY CONDITIONS

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ABSTRACT

Copulation and egg capsule deposition of *Thais haemastoma canaliculata* (Gray) and subsequent development of embryos to hatching were investigated. Adult *T. haemastoma canaliculata* deposited egg capsules, each containing approximately 3200 fertilized eggs. The number of capsules deposited by any one snail over several days varied between 20-30. The expected ontogeny of spiralean cleavage followed by gastrula, trochophore, and veliger larva occurred. The trochophore and veliger stages were easily distinguished from each other. No nurse eggs occur in this species. Hatching of planktotrophic veligers occurred within 13 days after capsule deposition at 25‰S and 25-26°C. Capsule wall dry weight decreased significantly; whereas, capsule content dry weight increased during the intracapsular period, largely due to increased calcification of embryonic shells. Embryonic calcium levels increased 24 fold during the intracapsular period.

The Southern Oyster Drill *Thais haemastoma canaliculata* (Gray) (=*T. haysae*, Clench, 1927) (Abbott, 1974), is a muricid gastropod inhabiting estuaries along the Louisiana gulf coast. This species is the primary predator on the Eastern Oyster *Crassostrea virginica* (Gmelin), the only commercially important species of oyster in Louisiana. It is believed that *T. haemastoma canaliculata* represents the greatest hazard to the survival of *C. virginica* (Pollard, 1973), thus making the drill an economically important destructive agent to the oyster fisheries in Louisiana (St. Amant, 1938, 1957; Burkenroad, 1931). In recent years, salt water intrusions, caused by the dredging of the Mississippi River at the Gulf of Mexico, have allowed *T. haemastoma canaliculata* to migrate further into the oyster seed grounds thus reducing the economic feasibility of extensive oyster culture (Pollard, 1973; Van Sickle *et al.*, 1976; Smith, 1983). The predation of *T. haemastoma canaliculata* on oysters and the regenerative ability of its feeding mechanism in response to injury have been previously described (Garton and Stickle, 1980; Roller *et al.*, 1984). Seasonal changes in the reproductive component weights of the southern oyster drill indicate major episodes of capsule deposition occurring between April and August (Belisle and Stickle, 1978).

Considerable interest in the reproductive biology and embryology of prosobranch gastropods has stimulated research by various investigators for many years. These investigations have varied from complete descriptions of the embryological development of certain gastropods (Conklin, 1897; Pelseneer, 1911; D'Asaro, 1966) to descriptions of specific morphological and ecological relationships of various larval forms (Thorson, 1950; Mileikovsky, 1971; Fretter, 1972; Spight, 1977; Strathmann, 1980; Hadfield, 1984; Pechenik, 1984). St. St. Amant (1938) provided a well written account of the general biology of *Thais floridana haysae* (Clench) (= *T. haemastoma canaliculata*); however, very few figures were included in the work, and the thesis was never published. D'Asaro (1966), using light microscopy, gave an excellent discussion of the embryogenesis of *Thais haemastoma floridana* (Conrad). Belisle and Byrd (1980) used electron microscopy to investigate *in vitro* egg activation and development through hatching in *Thais haemastoma*. No investigation to date has attempted to combine the use of light and scanning electron microscopy (SEM) to view the copulation, ovipositioning, capsule structure, and developmental stages of *T. haemastoma canaliculata*. Furthermore, intracapsular weight changes prior to hatching have not been investigated. Knowledge of embryonic weight changes prior to hatching would yield valuable information concerning possible nutritive contributions of intracapsular components.

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While considerable ambiguity exists concerning the exact taxonomic position and classification of *Thais* spp. of the Gulf of Mexico (Butler, 1985), the species examined in the present investigation was identified as *T. haemastoma canaliculata* (Gray) based on the presence of a large nodular shell possessing a strongly indented suture (Abbott, 1974). The objectives of the present investigation were to (1) observe copulation and capsule deposition of adult *Thais haemastoma canaliculata* in the laboratory; (2) determine the intracapsular developmental rate of embryos to hatching at a salinity (25‰) and temperature (25°C) similar to that experienced in the estuary; (3) examine changes in capsule structure and composition during development; and (4) rear hatched veligers.

MATERIALS AND METHODS

Adult *Thais haemastoma canaliculata* (shell length > 40 mm) were collected monthly during 1982 and 1983 from Bay Champagne near Grand Isle, Louisiana, U.S.A. Snails were transported to the laboratory and placed into 38 l aquaria (30 snails/aquarium) containing artificial seawater (Instant Ocean® Sea Water Mix) at the temperature and salinity of the collection site (at time of collection). The seawater near Grand Isle fluctuates in salinity and temperature between 10 and 35‰ and 10 and 30°C, respectively, over the course of a year (Barrett, 1971); however, the aquaria were maintained at constant salinity and temperature during this investigation. The male:female ratio in each aquarium was approximately 1:1. The snails were maintained on a photoperiod similar to the natural conditions under which they were collected. Drills were fed oysters (*Crassostrea virginica*) and clams [*Rangia cuneata* (Sowerby)].

Copulation and capsule deposition in the aquaria were observed and photographically recorded. Capsules were removed from the aquaria as soon as possible. Since the capsules were covered by the foot of the snail during deposition it was often necessary to delay their removal from the aquaria for several hours.

Individual egg capsules of known age were transferred to separate, clean glass culture bowls (10 cm tall x 19 cm diameter) containing filtered (0.45 μm) seawater at the appropriate temperature and salinity. The seawater in each bowl was aerated and changed daily throughout the experiment. Five capsules were sampled daily for the determination of developmental rates. Iridectomy scissors were used to open the egg capsules. The embryos were removed with a pasteur pipet and placed on glass slides with clay-supported coverslips. Embryos were then examined and photographed with a Leitz Wetzlar Orthoplan compound microscope with an Orthomat camera attachment. Embryos obtained from individual capsules were examined to determine if development to hatching was synchronous within a particular capsule. Intact and opened capsules were photographed with a Wild TYP stereo-dissection microscope with a Nikon M35-S camera attachment. Intracapsular osmolarity was determined with a Wescor vapor pressure osmometer.

Two days after deposition, ten capsules were opened and the embryos were removed and counted. Approximately one day prior to hatching, 10 randomly selected capsules from each culture bowl were dissected for mortality determination.

Each culture bowl was examined daily for hatched veligers, which were then transferred to additional culture bowls containing freshly aerated and filtered sea water (1 larva/100 ml). The water in each bowl was replaced daily. Veligers were then fed 10^4 cells/ml (final concentration) daily of *Isochrysis galbana* (Parke) - *Monochrysis lutheri* (Droop) (1:1). Algae were cultured using the method of Guilliard (1975).

For scanning electron microscopy (SEM), embryos were removed from the capsules for fixation. Veligers were first anesthetized with $MgSO_4$ and then fixed for SEM. The best anesthetization was achieved by slowly adding small amounts (approximately 0.1g) of granular $MgSO_4$ to the culture water until the larvae were completely immobile but had not contracted or withdrawn into their shells. Specimens were fixed overnight with 2.5% gluteraldehyde in 0.2M sodium cacodylate-sucrose buffer (731 mOsm; pH = 8.0). The sucrose was used to adjust the osmolality of the fixative to the appropriate salinity of the culture in order to reduce osmotic stress during fixation. After fixation, the specimens were rinsed in three changes of distilled water to remove all buffer salts, dehydrated in acidified 2,2-dimethoxypropane (DMP), and transferred to modified Beem™ capsules with a 25 μm Nitex screen over each end. The specimens were then critical-point dried in CO_2, coated with approximately 200 Å of Au/Pd, and examined with a Hitachi S-500 scanning electron microscope at 25 KV. Empty egg capsules were sectioned with a razor blade and prepared as above for SEM investigation. For light microscopy, intact capsules containing embryos and larvae were fixed overnight in formalin-acetic acid-alcohol (FAA), dehydrated in ethanol, cleared with xylene, embedded in paraffin, sectioned at 7 μm, and stained with Azan (Humason, 1972).

For capsule dry weight analysis, random samples of 20 capsules were taken one day after deposition (Day 1) and three days prior to hatching (Day 10). The total length of each capsule was measured with a vernier caliper. Each capsule was briefly rinsed in distilled water and then dissected into two components: capsule wall and capsule contents (embryos and albumen). The components were then lyophylized and capsule wall dry weight, capsule content dry weight, and total capsule dry weight was determined to 0.001 mg using an analytical balance. Capsule component indices were then calculated by the method of Stickle (1973). The relationship between capsule length and dry weight was analyzed by simple linear regression (SAS Institute Inc., 1985a, b). Differences between Day 1 and Day 10 dry weight components were compared by a two-sample t-test (Steel and Torrie, 1980).

Embryonic calcium levels were analyzed by atomic absorption spectrophotometry (Perkin-Elmer Corp., 1982). Twenty capsules on Day 1 and Day 10 were dissected and the contents were incubated in 10 ml of a 1% LaO_3 / 5% HCl mixture (40°C) for 1 hour to mobilize any calcium present. The contents of each capsule were then centrifuged. The supernatant was removed, diluted 2X with fresh LaO_3-HCl, and analyzed. Total inorganic material was determined on an additional sample of 20 capsules by ashing at 450°C for 4 hours.

Total organic material was calculated by subtracting the total ash (inorganic) from the pre-combustion dry weight. Day 1 and Day 10 calcium, organic, and other inorganic levels were compared by a two-sample t-test (Steel and Torrie, 1980).

RESULTS

COPULATION AND CAPSULE DEPOSITION

Copulation in the drills was observed in the field from late April to late June, 1982 and from late April to early June, 1983. During these months snails were found in large breeding aggregations which extended from approximately 0.5 m above the water surface at low tide to 1 m in depths. The number of snails comprising each aggregation varied from 6 to 27 individuals. Drills collected in early June, 1983 began copulating in the laboratory within 5 days. The duration of copulation was variable, lasting from approximately 2.5 hours to 3 days. During copulation the male crawled onto the shell of its partner and inserted its penis into the right side of the mantle cavity. Spermatozoa and prostatic secretions were presumably discharged into the genital aperture of the female (Fretter and Graham, 1962).

Egg capsule deposition occurred as early as six hours and up to sixty days after copulation was observed. In the laboratory, the egg capsules were attached to the glass walls of the aquaria, usually near the exhalant port of the undergravel filter system. Rarely were capsules deposited on oyster shells; however, oysters covered with *Thais* egg capsules have been collected from Grand Isle. Capsule deposition was intermittent. Snails were observed to cease deposition for a while, feed on oysters, and then resume deposition, sometimes in an entirely different location. Snails tended to attach their capsules together forming one large communal mass. The intermittent feeding behavior as described above and the communal egg masses made distinguishing which female laid specific capsules difficult. The number of capsules obtained from any one snail varied; however, most drills deposited 20-30 capsules in a mass. The duration of capsule deposition also varied, from as short as 2-3 hours to as long as 6-7 days. Snails were also observed to pause during deposition and remain on the capsule mass without feeding for several hours before resuming capsule laying.

Capsules were usually attached by their bases (Fig. 1), and formed a single layer on the substratum. In several cases capsules were observed attached together at various locations along their lengths; however, attachment never obstructed the opercular opening of any capsule in a mass. Butler (1954) reported similar findings.

The egg capsules of *Thais haemastoma canaliculata* are similar to those of *T. haemastoma floridana* as described by D'Asaro (1966). The capsules are somewhat conical in appearance, possessing a broad flat apical plate and tapering down to the base where they are typically attached to the substratum (Fig. 1). Each capsule possesses a convex and concave side along most of its length, giving the capsule an oblong appearance in cross section at the distal end (Fig. 2). However, the capsule is more circular in cross-section at its

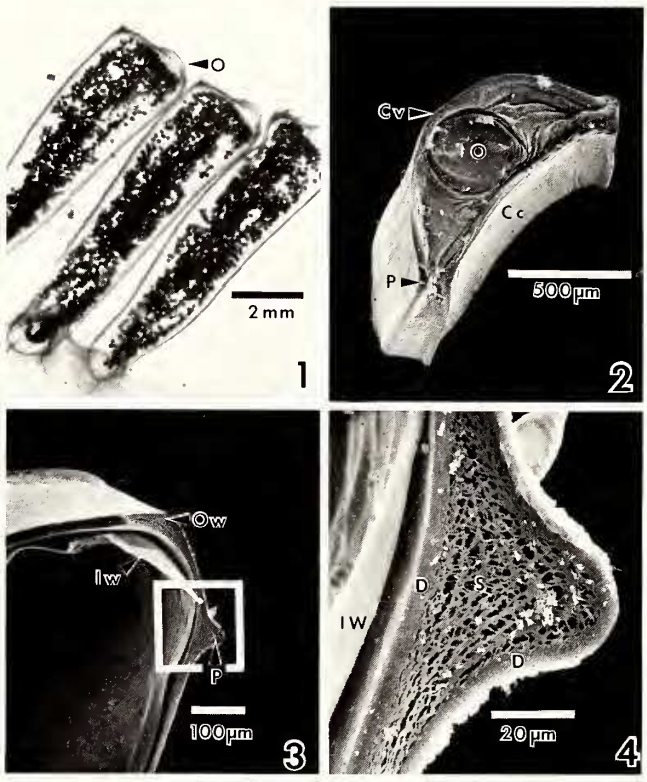


Fig. 1. Light micrograph of typical *Thais haemastoma* egg cases containing embryos. Hatching occurred approximately three days later (O, operculum). **Fig. 2.** Scanning electron micrograph (SEM) of an opercular view of an egg capsule (Cc, concave wall; Cv, convex wall; O, opercular plug; P, one lateral protuberance). **Fig. 3.** SEM of capsule cross-section showing both inner and outer walls (Iw, inner capsule wall; Ow, outer capsule wall; P, lateral protuberance). **Fig. 4.** SEM of capsule protuberance outlined in (3) (D, lateral dense layers of outer capsule wall; Iw, inner capsule wall; S, medial spongy mass of outer capsule wall).

tapered base. Four longitudinal ridges (2 on each side) separate the convex and concave sides. The two ridges on each side merge at the apical plate forming a lateral protuberance (Figs. 2-4). Each capsule is composed of a thick fibrous-appearing outer wall and a thin membranous inner wall, which readily separate during microscopical preparation (Fig. 3). The entire outer capsule wall appears to be composed of two compact, dense lateral layers and a spongy-fibrous medial layer (Fig. 4). The protuberances and ridges represent sculpturing of the outer wall only and do not make up any portion of the inner wall, which encloses the embryos and the nutritive albumen. A round, discoidal opercular plug is located on the apical plate at the distal end of each capsule (Fig. 2). The operculum swells and bulges outward a few days prior to hatching. At hatching the operculum disintegrates leaving a prominent opercular scar.

Capsule length varied from 0.84-1.13 cm ($\bar{x} \pm$ S.E.= 0.95 ± 0.01 cm; N=40). Capsule wall and capsule content dry weight varied from 0.47-1.57 mg ($\bar{x} \pm$ S.E.= 1.05 ± 0.05 mg; N=40) and from 0.14-1.20 mg ($\bar{x} \pm$ S.E.= 0.54 ± 0.04

mg; N=40) respectively. The total capsule dry weight varied from 0.92-2.14 mg ($\bar{x}$ ± S.E.= 1.60 ± 0.06 mg; N=40). Capsule wall and content dry weight comprised 65.6 ± 2.3 and 34.4 ± 2.3 ($\bar{x}$ ± S.E.) percent, respectively, of the total capsule dry weight. Capsule wall dry weight varied directly with capsule length: dry weight (mg)= −2.54 + (3.79 X length in cm) (r^2=0.673; N=40; P<0.001). A significant linear regression of total capsule dry weight on length also existed and is given as dry weight (mg)= −1.48 + (3.28 X length in cm) (r^2=0.468; N=40; P<0.001). No significant relationship existed between capsule content dry weight and capsule length (P>0.05). Each capsule contained 3246 ± 21 ($\bar{x}$ ± S.E.; N=10) embryos embedded in a viscous, albuminous fluid. Capsules, when deposited, were a milky white color, which during development turned light tan and finally dark brown just prior to hatching. Only three capsules deposited in the laboratory developed the dark purple color, characteristic of dead or stressed embryos (St. Amant, 1938; D'Asaro, 1966; Spight, 1977; Pechenik, 1982; Butler, 1954, 1985). Examination of these capsules revealed that all embryos were dead.

DEVELOPMENTAL RATE AND STAGES

Development of *Thais haemastoma canaliculata* was synchronous within a particular capsule throughout the entire period of encapsulation and required 12-13 days to hatching at 25‰S and 25ºC (Table 1). Unfertilized eggs were spherical and approximately 65-70 μm in diameter; however, as reported previously (St. Amant, 1938; D'Asaro, 1966), the majority of the yolk (deutoplasm) was concentrated in one pole (vegetal) with other cytoplasmic constituents being concentrated at the opposite (animal) pole. First and second polar body formation was complete within 2.5 hours after deposition of the capsule. By the second polar body stage (Fig. 5), the fertilized egg had elongated and the animal and vegetal areas were easily distinguished. The round yolk granules in the vegetal area were visible in live and preserved (Figs. 5, 6) zygotes. Early cleavage was restricted to the animal pole of the embryo. The first cleavage, producing the AB and CD blastomeres (Fig. 7) occurred 5-6 hours after deposition (Table 1). The second cleavage (Fig. 8) occurred within 2-4 hours after the first cleavage. As D'Asaro (1966) showed for *T. haemastoma floridana*, we found that the D blastomere possessed a large polar lobe (Fig. 8). Within 17-19 hours after capsule deposition, the 18 cell stage was complete. By that time, the polar lobe had been resorbed, and the large 2D macromere was seen (Fig. 9).

A stereoblastula containing a narrow segmentation cavity, as reported by St. Amant (1938), formed approximately 9-11 hours after polar lobe resorption (Table 1). Gastrulation by epiboly and archenteron formation (Fig. 10) was

Table 1. Developmental rate of *Thais haemastoma canaliculata* at 25‰S and 25-26ºC.

Developmental Event	Time
Fertilized egg with 2 polar bodies	2.5 hours
First cleavage	5-6 hours
Second cleavage	8-9 hours
16 cell stage	17-19 hours
Stereoblastula	28 hours
Early gastrula	3.5-4 days
Stomodael invagination, cephalic expansion & shell gland formation	5 days
Trochophore	5.5-6 days
Early veliger	7 days
Hatching	13 days

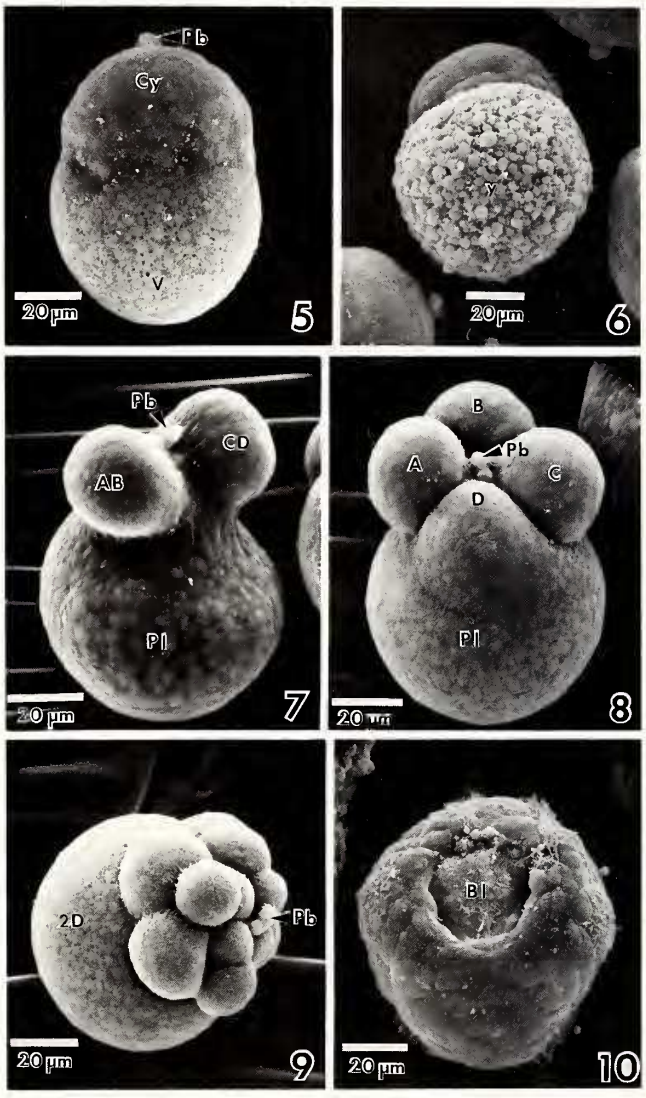


Fig. 5. SEM of fertilized egg after second polar body formation (Cy, cytoplasmic (animal) pole; Pb, polar bodies; V, vegetal yolk-containing pole). **Fig. 6.** SEM of vegetal view of ruptured polar lobe, illustrating dense yolk mass (y, yolk mass). **Fig. 7.** SEM showing first cleavage of the ovum, resulting in formation of AB and CD cells (Pb, polar bodies; Pl, polar lobe). **Fig. 8.** SEM of four-cell stage showing completion of A, B, C, and D cells with polar lobe (Pl) and polar bodies (Pb) still evident. **Fig. 9.** SEM of 2D cell, after polar lobe resorption (Pb, polar bodies). **Fig. 10.** SEM of gastrula stage, illustrating the blastopore (Bl).

observed within 3.5-4 days after oviposition. Stomodaeal invagination, cephalic expansion, and formation of the shell field invagination (Fig. 11) occurred 5 days after deposition and followed the same pattern as described for *Thais haemastoma floridana* (D'Asaro, 1966).

The early trochophore (Fig. 12) was characterized by a prominent stomodaeum, an apical tuft, the beginning of prototrochal and telotrochal ciliation, and the appearance of the larval kidneys. The late trochophore stage (Fig. 13) exhibited antero-posterior elongation, prominent larval kidneys, and well formed prototrochal, metatrochal, and telotrochal ciliation. The early veliger stage was characterized by the presence of the velar ciliation (Fig. 14). The dorsal margin of the shell gland was complete, and the protoconch covered the posterior region of the digestive gland's primordial cells. At this stage, the operculum was first evident (Fig. 15). By 8 days after capsule deposition, torsion, which results in a 180° rotation of the visceral mass, was complete. At this time, the apical ciliation and operculum were well developed, and the ventral foot and larval tentacles were first seen (Figs. 16-18).

No nurse eggs, as described by Rivest (1983), were observed. The viscosity of the intracapsular contents declined over the course of the developmental period; however, the measured intracapsular osmolarity did not change during development. It is therefore possible that the intracapsular albumen is consumed by the embryos and replaced by sea water.

CAPSULAR CONTENT CHANGES DURING DEVELOPMENT

Capsule weight changes prior to hatching are illustrated in Table 2. During the intracapsular developmental period, the weight of the capsule contents significantly increased 63.0%; capsule wall weight decreased 43.4%; and the total capsule weight (contents and wall) decreased 18.4%. Total capsule ash significantly increased 37.8%, while total capsule calcium increased 24-fold over the encapsulated developmental period. Total capsule organic material significantly decreased 37.7%; however, other inorganic material (excluding calcium) showed a non-significant increase of 2.0%.

HATCHING AND REARING OF VELIGERS

Hatching of veligers (Figs. 17, 18) at 25‰S and 25°C occurred between 12-13 days after capsule deposition. The shell length at hatching was 49.7 ± 8.3 μm. Hatching was accomplished through the dissolution of the capsule's operculum, possibly by mechanical means (St. Amant, 1938) or by chemical means (Sullivan and Bonar, 1984). Most (96-100%) embryos developed into normal appearing veligers and survived to hatching. In some capsules approximately 2-4% of the veligers were either dead or malformed at hatching. Hatched veliger larvae survived up to 50-53 days when kept in laboratory cultures and fed a mixture of *Isochrysis galbana* and *Monochrysis lutheri*. Ninety percent of the hatched veligers survived 45-50 days in culture. The shell length of the veligers after 37 days in culture was 122.4 ± 28.3 μm. No settlement/metamorphosis occurred, even though the larvae appeared healthy and fed on the algal species provided (Fig. 18).

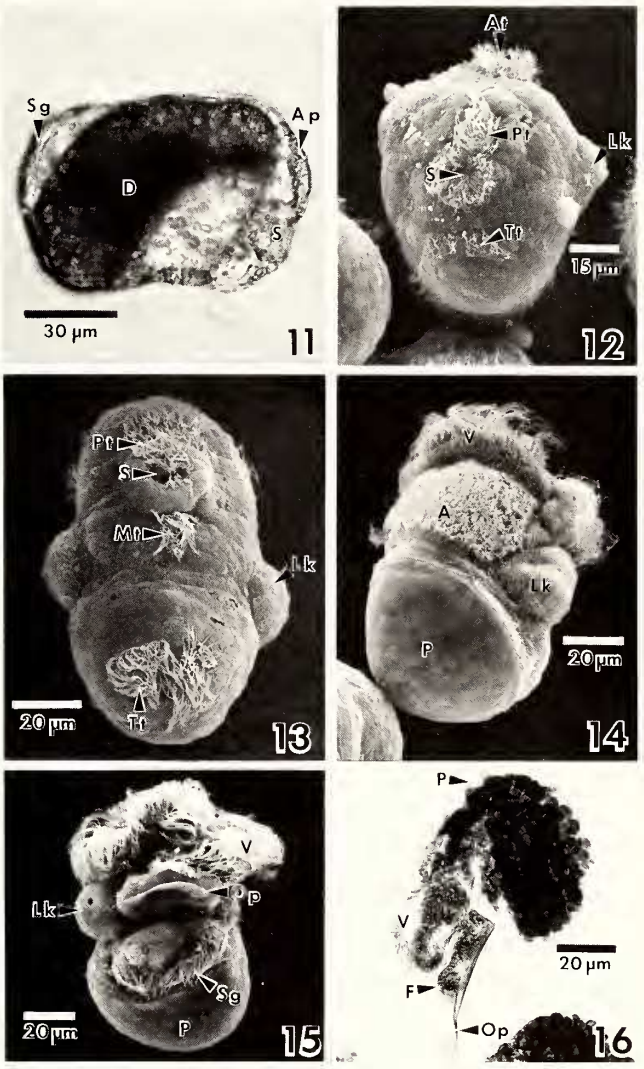
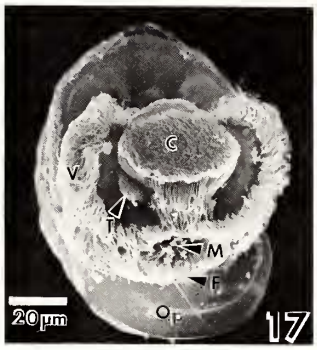


Fig. 11. Light micrograph showing stomodael invagination (S) and apical plate formation (Ap), immediately after gastrulation and prior to formation of trochophore (D, digestive system primordium; Sg, shell gland). **Fig. 12.** SEM of early trochophore stage (At, Apical tuft ciliation; Lk, larval kidney; Pt, prototrochal ciliation; S, stomodaeum; Tt, telotrochal ciliation). **Fig. 13.** SEM of late trochophore stage, showing formation of metatrochal ciliation (Lk, larval kidney; Mt, metatrochal ciliation; Pt, prototrochal ciliation; S, stomodaeum; Tt, telotrochal ciliation). **Fig. 14.** SEM of a dorsolateral view of early veliger larva (A, Apical ciliation; Lk, larval kidneys; P, protoconch; V, velum). **Fig. 15.** SEM illustrating a ventral view of an early veliger larva, illustrating shell operculum formation and prominent shell gland ciliation (Lk, larval kidneys; Op, shell operculum; P, protoconch; Sg, shell gland; V, velum). **Fig. 16.** Light micrograph illustrating a midsaggital section (7 μm) through a veliger (three days prior to hatching), showing further elongation of foot and operculum (F, foot; Op, shell operculum; P, protoconch; V, velum).

Table 2. Capsule component weights on Day 1 and Day 10 for *Thais haemastoma canaliculata* capsules. Capsule components (in mg) are separated into organic, Ca^{2+}, and other inorganic components. N= 40 capsules.

	DAY 1	DAY 10	T VALUE
CAPSULE CONTENTS			
Organics	0.090 ± 0.005*	0.181 ± 0.004	14.84‡
Calcium	$5.60 \times 10^{-4} \pm 5.2 \times 10^{-6}$	0.161 ± 0.003	55.46‡
Other			
Inorganics	0.323 ± 0.012	0.332 ± 0.009	0.56 N.S.
Total	0.414 ± 0.044	0.675 ± 0.056	3.64‡
CAPSULE WALL			
Organics	1.218 ± 0.031	0.634 ± 0.039	11.69‡
Calcium	$6.41 \times 10^{-3} \pm 1.6 \times 10^{-4}$	$7.21 \times 10^{-3} \pm 1.0 \times 10^{-4}$	4.14‡
Other			
Inorganics	0.119 ± 0.004	0.119 ± 0.004	0.03 N.S.
Total	1.344 ± 0.033	0.760 ± 0.038	11.61‡
CAPSULE TOTAL (wall and contents)			
Organics	1.309 ± 0.056	0.815 ± 0.077	5.18‡
Calcium	$6.97 \times 10^{-3} \pm 8.9 \times 10^{-5}$	0.168 ± 0.003	54.46‡
Other			
Inorganics	0.443 ± 0.012	0.452 ± 0.010	0.56 N.S.
Total	1.759 ± 0.054	1.435 ± 0.001	3.29†

* - Mean ± S.E.
‡ - Statistically significant at $\alpha = 0.001$
† - Statistically significant at $\alpha = 0.01$
N.S. - Nonsignificant



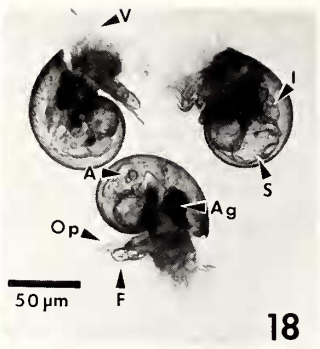


Fig. 17. SEM showing an anterior view of *a* hatched veliger, illustrating *a* well developed velum (V), larval tentacle (T), and cephalic ciliation (C) (F, foot; M, mouth; Op, shell operculum). **Fig. 18.** Light micrograph of newly hatched veligers, illustrating prominent structures (A, algal cell in gut; Ag, anal gland; F, foot; I, intestine; Op, shell operculum; S, stomach; V, velar cilia).

DISCUSSION

We observed, with the aid of scanning electron microscopy, a distinct trochophore stage (Figs. 12, 13) for *Thais haemastoma canaliculata*. St. Amant (1938) had earlier reported that in *T. floridana haysae* the trochophore was atrochal and could not be distinguished from the early veliger stage; therefore, the early veliger could be identified only after the shell was formed. The development of the velum (Fretter and Graham, 1962), which is difficult to observe using standard light microscopy (St. Amant, 1938), is easily seen using SEM techniques (Figs. 12-15). The development of the protoconch is also more apparent from SEM observations (Fig. 14). St. Amant was unable to identify the onset of torsion in this species. We found that in *T. haemastoma canaliculata* torsion occurs prior to hatching, which agrees with D'Asaro's (1966) observations of *T. haemastoma floridana* development.

Belisle and Byrd (1980) identified two different cleavage patterns occurring in *Thais haemastoma*, as opposed to the one distinct pattern reported by St. Amant (1938) and D'Asaro (1966). In the present study, we failed to observe the second cleavage pattern observed by Belisle and Byrd (1980). The only cleavage pattern we observed agrees with that reported by D'Asaro (1966). The absence of the second cleavage pattern in our investigation does not deny its existence. This second pattern could be an infrequent deviation from the "normal" pattern usually observed.

The difference in developmental rate observed in the present study (13 days to hatching at $25^0/_{00}$S and 25-26°C) and that observed by Belisle and Byrd (1980) (16 days to hatching at $20^0/_{00}$ and 24°C) could be due to differences in experimental temperature and salinity. Belisle and Byrd (1980) did not specify the subspecies of snail they studied. The developmental patterns and rates we observed for *Thais haemastoma canaliculata* at $25^0/_{00}$S and 25-26°C are very similar to those reported for *T. haemastoma floridana* by D'Asaro (1966). The ranges of these two subspecies overlap and both are found on the Louisiana coast, although *T. haemastoma canaliculata* is more numerous (St. Amant, 1938). Butler (1954) made reciprocal crosses between the two subspecies and obtained normal larval development, suggesting that hybridization could occur in this area. Since the embryology of *T. haemastoma canaliculata* and *T. haemastoma floridana* is similar (St. Amant, 1938; Butler, 1954; D'Asaro, 1966; present study), the separation of the two into separate subspecies based on shell morphology alone is possibly unjustified. Further data, in the form of electrophoretic analysis, are needed.

Hatching of veligers, in the present study, occurred between 12-13 days after oviposition and was possibly accomplished by chemical dissolution of the capsule operculum, as occurs in the mud snail *Ilyanassa obsoleta* (Say) (Sullivan and Bonar, 1984). Veligers in laboratory culture survived 50-53 days after hatching but did not metamorphose. Algal cells were observed in the gut of the veligers (Fig. 18) and the larvae appeared healthy; however, none survived to settlement and metamorphosis. It is possible that the veligers did not obtain enough nutrients from the algal cells provided. The veligers did survive an extended time (50 days) and showed evidence of some growth (from 49.7 μm to 122.4 μm). Furthermore, the algal species and concentration provided have been sufficient for other planktotrophic larvae (Ament, 1979; Jespersen and Olsen, 1982; Sprung, 1984); however, the nutrient levels and quality necessary for maintenance could possibly not be sufficient for growth and metamorphosis. Hadfield (1984) showed that a high degree of substratum chemical

specificity can be required to induce settlement and metamorphosis in molluscan larvae. Several chemical compounds, from naturally occurring substances, have been identified as inducers of larval settlement and metamorphosis (Morse *et al.*, 1979; Heslinga, 1981; Rumrill and Cameron, 1983; Morse and Morse, 1984). It is possible that one or more inducers exist for *Thais haemastoma canaliculata*. Such inducers could exist in encrusting algae on oyster shells or possibly in polychaete tubes or barnacles upon which young *Thais* prey. Oysters, tube-dwelling polychaetes, and barnacles are abundant along the Louisiana coast.

Gastropod species possessing teleplanic veligers could be dispersed over a large geographic range and would have a planktonic existance of long duration (Scheltema, 1978). It appears from our results and the observations of others (St. Amant, 1938; D'Asaro, 1966; Scheltema, 1978) that *Thais haemastoma canaliculata* veligers are teleplanic and are likely to survive as long in the field as they did in the laboratory.

The spongy/dense layering of the outer wall of the egg capsules could just be the result of the process used to form the capsule and have no specific function; however, in our opinion this layering appears similar to that seen in vertebrate long bones (Mader, 1985) and could possibly aid in lending strength and support to the capsules thus protecting the enclosed embryos against physical damage. The protuberances and ridges could aid in maintaining an upright capsule and further enhance the protection of the delicate embryos inside.

Egg capsule dry weight varied directly with capsule length ($r^2 = 0.673$; $P < 0.001$) for *Thais haemastoma canaliculata*. The dry weight of individual capsule components varied differently from ovipositioning to hatching. The overall decrease in total capsule dry weight during the intracapsular developmental period (Table 2), possibly reflects the loss of metabolic end products through the capsule wall. The weight of a single capsule operculum (unpublished data) is only 0.08 $\pm$ 0.02 mg ($N = 20$); therefore, the 43.4% decrease in capsule wall weight appears too high to be explained solely by chemical dissolution of the opercular cap. It is possible that portions of the inner matrix of the capsule wall are eroded prior to hatching; however, in this investigation all hatched veligers exited from the capsule through the operculum. It is therefore unlikely that erosion of other portions of the capsule wall would aid the hatching process by forming multiple exits. It is possible that nutrients or other substances are removed from the wall and utilized by the developing embryos. Since the majority of the capsule wall is composed of organic material (Table 2) and the uptake of dissolved organic material (DOM) by molluscan larvae has been documented (Manahan, 1983), this hypothesis is possible. This hypothesis could also aid in explaining the doubling of the capsule content organic weight; however, this is speculative since we have no confirming data. The dry weight of the capsule contents (albumen and embryos) significantly increased 63% prior to hatching. We have shown (Table 2) that much of this increase (24%) is due to the uptake of calcium by the embryos, presumably for calcification of the shell prior to hatching. Eyster (1986) showed that calcium was the main constituent of early shell mineralization for several species of gastropod veligers. Likewise, our data for *T. haemastoma canaliculata* support an observed overall increase in calcium content of these veligers prior to hatching. We found a small amount of calcium associated with the capsule wall (Table 2), which was probably due to residual calcium adsorbtion to the wall, or possibly a small number of embryos that we neglected to remove. None of the increase in capsular content dry weight (i.e. embryonic weight) was due to inorganic materials other than calcium. It is expected of marine organisms with planktotrophic larvae that most of the organic growth should occur after hatching and during the planktonic existence (Scheltema, 1967; Pilkington and Fretter, 1970; Pechenik and Fisher, 1979; Pechenik, 1980, 1984; Pechenik and Lima, 1984). We observed a significant doubling in the organic material of the capsule contents; however, we made no weight measurements of planktonic veligers. The increase in the organic material of the capsule contents could be related to the corresponding loss of organic material from the capsule walls; however we have no data to prove this assumption. It is clear that observations on growth and weight changes of planktonic stages is needed before any comparisons can be attempted.

It was the purpose of this study to add to earlier investigations of *Thais* developmental patterns, egg capsule structure, and weight changes over the course of intracapsular development. Our findings and those of St. Amant (1938), Butler (1954), D'Asaro (1966), and Belisle and Byrd (1980) illustrate the tremendous reproductive potential for this species. Even though the planktonic larval mortality must be quite high, when one considers the sheer number of embryos contained in a single capsule (about 3200), the number of capsules deposited by a single female (20-30), and the 96-100% survival to hatching (laboratory conditions), it is no surprise that this species is a serious economic threat to the oyster industry along the United States gulf coast.

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